Biol Blood Marrow Transplant 21 (2015) 755–760

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Bloodstream Infections in Adult Patients Undergoing Cord Blood Transplantation from Unrelated Donors after Myeloablative Conditioning Regimen

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Article history:
Received 8 November 2014
Accepted 31 December 2014

Key Words:
Bloodstream infections
Umbilical cord blood transplantation
Engraftment
Nonrelapse mortality

A B S T R A C T

The incidence, epidemiology, and risk factors of bloodstream infection (BSI) and their impact on transplant outcomes after umbilical cord blood transplantation (UCBT) are not well defined. Between May 1997 and December 2012, 202 isolates in 189 episodes of BSI were registered in 134 of 241 patients who underwent single-unit myeloablative UCBT. Cumulative incidence (CI) of developing at least 1 episode of BSI was 21%, 29%, 34%, 42%, and 52% at days +7, +14, +30, +100, and +365, respectively. The median time of onset for the first BSI episode was day +10 (range, day −7 to +1217). Early BSI before day 7 was associated with increased nonrelapse mortality (relative risk [RR], 1.5; 95% confidence interval [CI], 1.1 to 2.3; $P = .04$), whereas BSI before day 14 was an independent adverse risk factor for neutrophil recovery (RR, .6; 95% CI, .5 to .9; $P = .002$). A higher $CD8^+$ cell dose of the graft was the only variable independently associated with reduced risk of BSI (RR, .1; 95% CI, .02 to .7; $P = .02$). The gram-negative rod (GNR) to gram-positive bacteria ratio was .9 before day +30 and 1.6 thereafter ($P = .03$). *Escherichia coli* (31%) and *Pseudomonas* sp. (28%) were the most frequently isolated among GNR. The overall crude mortality rate was 12% at day 7 and was higher for GNR (18%) compared with gram-positive bacteria (7%) ($P = .03$). These findings emphasize the importance of preventing bacterial infections during conditioning and the very early post-UCBT period.

INTRODUCTION

Bloodstream infections (BSIs) are a leading cause of morbidity and mortality in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1,2]. In the umbilical cord blood transplantation (UCBT) setting, characterized by a delayed neutrophil recovery and immune reconstitution, management of infections is particularly important. Therefore, efforts to define the epidemiologic characteristics, outcome, and prognostic factors of BSI after UCBT are warranted.

Epidemiology and microbial resistance as well as the clinical characteristics of BSIs and their impact on outcome may vary with different transplant procedures and stem cell sources [3,4]. A better knowledge of these features is essential to guide early intervention with empirical antibiotic treatment. Although UCBT has been increasingly used over the past 2 decades, information on BSI in this specific scenario is rather scarce. As far as we know, only 3 studies from Japan have extensively evaluated this complication and were all restricted to the early post-transplant period [5-7]. Other reports on infectious complications after UCBT usually included a relatively low number of patients with few documented bacteremias [4,8-10]. In addition, the impact of BSI on transplant outcomes and the risk factors for development of this complication have not been definitely identified. Only 1 large registry-based study was able to

Financial disclosure: See Acknowledgments on page 760.

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http://dx.doi.org/10.1016/j.bbmt.2014.12.038
1083-8791/

demonstrate a negative effect of early BSI on survival [7]. The aim of this study was to analyze the incidence of BSI and the causative organisms, microbial resistance, clinical characteristics, outcome, and prognostic factors in a large series of adult patients undergoing single-unit myeloablative UCBT at a single institution.

METHODS

Patients

Between May 1997 and December 2012, 241 consecutive adult patients with hematologic malignancies underwent myeloablative UCBT from unrelated donors at our institution. The eligibility criteria, described in detail elsewhere [11,12], were as follows: high-risk hematologic malignancy; no suitable related donor; need for an urgent transplantation or lack of HLA-identical unrelated donor after searching in the international registries for no more than 3 months; and an available cord blood unit fulfilling minimum established criteria for both HLA compatibility between donor and recipient and cell dose. The institutional review board approved the clinical protocols, and written informed consent was obtained from all patients according to the declaration of Helsinki.

Conditioning Regimen and Management of Graft-versus-Host-Disease

Myeloablative conditioning regimens were all based on the combination of thiotepa, busulfan, cyclophosphamide, or fludarabine and antithymocyte globulin. For graft-versus-host disease (GVHD) prophylaxis, all patients received cyclosporine combined with either prednisone or mycophenolate mofetil. Patients developing acute GVHD received high-dose methylprednisolone as initial therapy (2 to 20 mg/kg/day), and antithymocyte globulin was used in refractory cases. Chronic GVHD was treated with prednisone (1 mg/kg/day). Details on dose and schedules were reported elsewhere [12-15].

Supportive Care and Management of Infections

Patients were nursed in high-efficiency particulate air–filtered rooms. Intravenous access was achieved with a double-lumen tunneled central venous catheter. Filgrastim was administered subcutaneously 5 μg/kg/day from day +7 until neutrophil recovery. All patients received oral ciprofloxacin prophylaxis (500 mg every 12 hours) until neutrophil recovery or initiation of broad-spectrum antibiotic therapy.

Pneumocystis jiroveci prophylaxis consisted of co-trimoxazole (320/1600 mg trimethoprim/sulfamethoxazole daily) from day −10 to day −2 and was then restarted after neutrophil recovery (2 days a week) to be maintained a minimum of 1 year or until stopping immunosuppression. Fluconazole prophylaxis (100 mg/day p.o.) was administered in some patients at the beginning of the study period. From November 2003, i.v. itraconazole (200 mg/day) was used. When oral intake was feasible, all patients received antifungal prophylaxis for 6 months or during steroid therapy with a mold-covering agent.

All blood products were irradiated and leukocyte depleted. Cytomegalovirus prophylaxis, infection surveillance, and treatment were described in detail elsewhere [15]. Nonspecific i.v. immunoglobulin was administered at a dose of 500 mg/kg weekly until day +100 and then monthly within the first year of transplantation.

In case of fever (body temperature ≥38°C) or other signs or symptoms of infection, patients were treated with empirical broad-spectrum i.v. antibiotics following an institutional protocol. At the beginning of the study period, 30 patients were initially treated with empirical i.v. antipseudomonal penicillin (piperacillin/tazobactam, 4/.5 g every 6 hours) in combination with amikacin (single daily dose of 20 mg/kg, maximum 1.5 g). From January 2002, empirical therapy was changed to carbapenem plus glycopeptide in the remaining 211 patients. Liposomal amphotericin B (3 mg/kg/day) or, more recently, caspofungin (70 mg initial dose, then 50 mg/day) was added if patients remained febrile for 5 days after the start of initial therapy. In patients with a microbiologically documented infection, therapy was modified, if necessary, according to susceptibility testing. Before the start of antibiotic therapy, samples of at least 2 sets of blood cultures (at 20-minute intervals) were collected through a 2-lumen catheter and peripheral vein. Samples were taken daily through the catheter if fever persisted. In patients receiving high-dose methylprednisolone, surveillance blood cultures were performed weekly.

Blood culture samples were processed using an automated BACTEC 9240 (Becton Dickinson, Sparks, NV) or BacT/Alert (Organon Teknika, Durham, NC) system to process. The central venous catheter was systematically removed in case of malfunction, thrombosis, documented candidemia, hemodynamic instability, tunnel infection, and for high suspicion of catheter-related bacteremia due to persistence of positive blood cultures despite the use of appropriate antibiotic therapy.

Definitions

Neutrophil recovery was defined as an absolute neutrophil count of $.5 \times 10^9$/L or greater on 3 consecutive days. Patients who survived more than 28 days after transplantation and who failed to achieve neutrophil recovery were considered as graft failures. Time to neutrophil recovery was defined as the time required to reach the first day of neutrophil recovery.

BSI occurring after disease relapse were excluded from the analysis. Single blood culture isolates were sufficient to consider BSI, regardless of the presence of a clinical site of infection, except for coagulase-negative staphylococci (CoNS) and *Corynebacterium* species other than *C. jeikeium*, and other common skin contaminants, which required at least 2 positive blood culture specimens. BSI was considered polymicrobial if ≥2 pathogens were isolated in the same blood culture or in separate blood cultures obtained in a period of 72 hours. Bacteremia occurring more than 14 days after a previous episode and separated by repeatedly negative blood cultures was considered to be another episode of BSI. Pathogens with intermediate susceptibility or resistance were considered as resistant. Enterobacteria producing extended-spectrum β-lactamases (ESBLs) were defined as strains with acquired enzymes capable of hydrolyzing and causing resistance to penicillins, cephalosporins, and monobactams (not cephamycins or carbapenems) inhibited by beta-lactamase inhibitors (like clavulanic acid or tazobactam). Susceptibility studies were done following Clinical Laboratory and Standards Institute procedures and breakpoints. Multidrug resistance was defined as acquired nonsusceptibility to at least 1 usually active agent in 3 or more antimicrobial categories following criteria proposed by Magiorakos et al. [16]. For the purpose of the study, the periods after transplantation were classified as early (from days 0 to +30), intermediate (from days +31 to +100), late (from days +101 to +365), or very late (after day +365).

Data Collection

Data of patients, transplant procedures, and infectious complications were prospectively collected in all patients undergoing UCBT and then settled in a computerized database. Clinical charts were additionally reviewed for inconsistent or missing data. Data collection for the analysis was stopped on January 1, 2013.

Statistical Analysis

The chi-square test with Yates' correction and Fisher's exact test were used for comparisons of categorical variables. Two-tailed *P* values were used. The probabilities of neutrophil recovery, nonrelapse mortality (NRM), and BSI were estimated by the cumulative incidence method (marginal probability) [17]. For cumulative incidence analyses of neutrophil recovery, death in complete remission was considered as a competing cause of failure, relapse was the competing event for NRM, and death and relapse were competing events for BSI. The Fine and Gray method for competing events [18] was used for multivariable analysis.

Variables considered for risk factor analysis were age; gender; recipient weight; recipient cytomegalovirus serology; disease status at transplantation; HLA compatibility; conditioning regimen; GVHD prophylaxis; type of empirical antibiotic therapy; total nucleated cell count; $CD34^+$, $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD56^+$, and $CD19^+$ cells of the graft; and BSI at days 7, 14, and 30. BSIs at these time points were considered as a time-dependent covariate. Correlation between variables was analyzed by Spearman's correlation test. Statistical analysis were conducted using R version 3.0.1 (The CRAN project) [19].

RESULTS

Patient Characteristics

Table 1 shows the main characteristics of the 241 adult patients who underwent myeloblative UCBT from unrelated donor. Median age was 34 years (range, 15 to 57), and 148 patients were males. The diagnosis were acute myeloid leukemia/myelodysplastic syndrome (40%), acute lymphoblastic leukemia (37%), chronic myeloid leukemia (12%), and a miscellaneous group of other underlying conditions (11%). One hundred thirteen cord blood recipients (48%) were transplanted in the early phase, whereas the remaining patients had a more advanced disease status. Median follow-up of surviving patients was 73 months (range, 10 to 180).

Cord Blood Unit and Transplant Characteristics

Table 1 shows the characteristics of cord blood units and those related to the transplant procedure. Except for 11

Table 1
Patient-, Graft- and Transplantation-Related Characteristics

Characteristic	Value
No. of patients	241
Age, yr	
Median	34
Range	15-57
Gender	
Male	148 (61)
Female	93 (39)
Diagnosis	
Acute myeloid leukemia/myelodysplastic syndrome	98 (40)
Acute lymphoblastic leukemia	90 (37)
Chronic myeloid leukemia	28 (12)
Chronic lymphoproliferative disorders	19 (8)
Other	6 (3)
Disease status at transplant	
Early	113 (48)
Intermediate	62 (25)
Advanced	66 (27)
Cytomegalovirus serologic status before transplantation	
Positive	189 (78)
Negative	52 (22)
HLA compatibility	
6 of 6	11 (5)
5 of 6	56 (23)
4 of 6	170 (70)
3 of 6	4 (2)
Conditioning regimen	
TT + BU + CY + ATG	72 (29)
TT + BU + FLU + ATG	170 (71)
GVHD prophylaxis	
Cyclosporine A + prednisone	164 (68)
Cyclosporine A + MMF	77 (32)
No. of nucleated cells infused, $\times 10^7$/kg	
Median	2.3
Range	1.0-5.8
No. of CD34$^+$ cells infused, $\times 10^5$/kg	
Median	1.3
Range	.08-21.1
No. of CD8$^+$ cells infused, $\times 10^6$/kg	
Median	1.6
Range	.5-6.8

Values are number of cases with percents in parentheses (percentages may not sum to 100 because of rounding), unless otherwise indicated. TT, thiotepa; BU, busulfan; CY, cyclophosphamide; ATG, antithymocyte globulin; FLU, fludarabine; MMF, mycophenolate mofetil.

patients who received a fully matched cord blood unit, the remaining 230 patients (95%) received an HLA-mismatched unit. Donor–recipient disparity in 1 and 2 of 6 antigens occurred in 56 patients (23%) and 170 patients (70%), respectively. Four additional patients (2%) received a 3-antigen mismatched unit. The median number of total nucleated cells and CD34$^+$ cells infused was 2.3 $\times$ 10^7/kg (range, 1.0 to 5.8) and 1.3 $\times$ 10^5/kg (range, .08 to 21.1), respectively.

Incidence and Timing of BSI

BSIs were documented in 189 episodes in 134 patients who underwent CBT. Ninety-two patients had 1 BSI episode (38%), 30 patients had 2 episodes (12%), 11 had 3 episodes (5%), and 1 patient had 4 episodes (1%). Cumulative incidence risk of developing at least 1 episode of BSI was 21% at day +7, 29% at day +14, 34% at day +30, 42% at day +100, 52% at day +365, and 54% at 4 years.

The median time of onset for the first BSI episode was day +10 (range, day −7 to +1217). Seventy-three BSIs (39%) were documented in 65 patients (27%) before neutrophil recovery. The distribution of BSI episodes by periods after transplantation was the following: 98 (52%) during the early period (median day, +6), including 3 that occurred during conditioning; 38 (20%) during the intermediate period (median day, +57); 41 (22%) during the late period (median day, +139); and 12 (6%) during the very late period (median day, +556).

Impact of BSIs on Transplant Outcomes

Neutrophil recovery

Twelve patients died before day +28 at a median time of 16 days (range, 9 to 24) without evidence of engraftment. Nine patients assessable for engraftment did not achieve neutrophil recovery and were considered as primary graft failures. Two hundred twenty patients experienced neutrophil recovery at a median time of 21 days (range, 9 to 57). The cumulative incidence of neutrophil recovery at 57 days was 92%. Early BSI before day 14 after UCBT showed a negative impact in univariable analysis (81% versus 96%; $P = .003$). In multivariable analysis, CD34$^+$ cell count (relative risk [RR], 1.1; 95% confidence interval [CI], 1.1 to 1.2; $P < .001$) and BSI before day 14 (RR, .6; 95% CI, .5 to .9; $P = .002$) remained as independent risk factors for neutrophil recovery.

NRM and causes of death

One hundred thirteen transplant-related deaths occurred at a median time of 114 days after transplantation (range, 7 to 3930). The primary causes of death were infection in 66 patients, GVHD in 19 patients, and a variety of transplant-related causes in the remaining 28 patients. Of the 66 deaths attributable to infection, 28 were bacterial infections, 14 invasive fungal infections, 15 viral infections, 3 mixed infections, 1 leishmaniasis, and 5 were not microbiologically documented. The cumulative incidence of NRM at day 100 and at 2 years was 20% and 45%, respectively. Early BSI before day 7 after UCBT showed a negative impact on 100-day NRM in univariable analysis (32% versus 16%; $P = .04$). NRM at day 100 was 21% and 25% ($P = .8$) for early gram-positive bacteria (GPB) and gram-negative rod (GNR) BSI before day 7, respectively. In multivariable analysis, diagnosis of acute leukemia (RR, .5; 95% CI, .3 to .7; $P < .001$) and BSI before day 7 (RR, 1.5; 95% CI, 1.1 to 2.3; $P = .04$) remained as independent risk factors for NRM.

Risk Factors for BSI

In univariable analysis, CD34$^+$ cell dose infused with a best cut-off at 1 $\times$ 10^5/kg ($P = .03$) and CD8$^+$ cell dose infused with a best cut-off at 1.6 $\times$ 10^6/kg ($P < .001$) influenced the risk of developing BSI. Multivariable analysis showed that a higher CD8$^+$ cell dose was the only variable independently associated with reduced risk of BSI (RR, .1; 95% CI, .02 to .7; $P = .02$). The R correlation coefficient between infused CD34$^+$ and CD8$^+$ cells was .41 ($P < .01$).

Etiology of BSIs

Overall, there were 202 isolates over 189 episodes of BSI. Seventy-six single BSI were due to GPB (40%), 87 to GNRs (46%), and 13 to fungi (7%). Among 13 polymicrobial BSIs (7%), 7 were due to GPB and GNRs, 3 to GNRs, 1 to GPB, 1 to GPB and *Candida*, and 1 to GNRs and *Candida*. Detailed etiology by post-transplant period is outlined in Table 2. The GNR/GPB ratio was .9 in the early period and 1.6 thereafter ($P = .03$) and did not change significantly during the observation period.

Gram-positive bacteria

Overall, 85 GPB were isolated (Table 2). The predominance of GPB before engraftment was mainly with CoNS,

Table 2
Bloodstream Isolates by Transplant Period

Organism	Early	Intermediate	Late	Very Late	All Periods
Total	102	43	45	12	202
Gram-positive	48 (47)	18 (41)	15 (33)	4 (33)	85 (42)
Staphylococcus coagulase-negative	29	12	11	4	56
Staphylococcus aureus	4	0	1	0	5
Enterococcus	4	5	2	0	11
Streptococcus viridans	7	0	0	0	7
Corynebacterium jeikeium	2	1	0	0	3
Streptococcus agalactiae	1	0	0	0	1
Streptococcus pneumoniae	1	0	0	0	1
Rhodococcus equi	0	0	1	0	1
Gram-negative	43 (42)	24 (55)	28 (62)	7 (58)	102 (51)
Escherichia coli	20	4	6	2	32
Klebsiella-Enterobacter-Serratia	2	2	5	0	9
Pseudomonas spp.	8	9	9	3	29
Stenotrophomonas maltophilia	5	5	1	0	11
Acinetobacter spp.	6	2	2	0	10
Salmonella spp.	0	1	1	2	4
Aeromonas hydrophila	1	0	0	0	1
Proteus mirabilis	0	0	1	0	1
Nontypified gram nonfermenter	1	0	2	0	3
Morganella morganii	0	0	0	1	1
Campylobacter spp.	0	1	0	0	1
Fungi	11 (11)	1 (2)	2 (4)	1 (8)	15 (7)
Candida albicans	1	0	0	0	1
Candida glabrata	1	0	0	1	2
Candida krusei	5	0	1	0	6
Candia parapsilosis	2	1	0	0	3
Candida tropicalis	1	0	1	0	2
Blastoschizomyces capitatus	1	0	0	0	0

Values in parentheses are percents.

which accounted for 66% of all GPB isolated, followed by *Enterococcus* sp. (13%), *Streptococcus viridans* (8%), and *Staphylococcus aureus* (6%). Eleven of 12 (92%) BSIs by *S. aureus* or *Streptococcus viridans* occurred in the neutropenic phase, whereas 7 of 11 (64%) enterococcal infections appeared after neutrophil recovery. Of these, 7 were due to *Enterococcus faecalis* and 4 to *Enterococcus faecium*. Only 1 isolate of *S. aureus* was methicillin-resistant. The distribution of GPB changed significantly during the observation period. CoNS, *Streptococcus viridans*, and *Enterococcus* species accounted for 80%, 2%, and 4% before 2007 and 50%, 15%, and 23% from 2007 onward, respectively ($P = .01$).

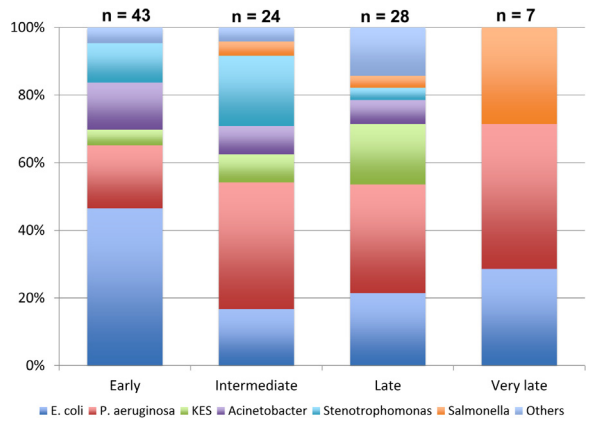

Figure 1. Etiology of BSIs caused by GNRs according to the post-transplant period.

Gram-negative bacteria

Among 102 GNRs (Table 2), *Escherichia coli* and *Pseudomonas* spp. were the most frequently isolated (31% and 28% of GNRs, respectively). *Stenotrophomonas maltophilia* and *Acinetobacter* spp. accounted for 11% and 10% of GNRs, respectively. Isolates had a different distribution according to the post-transplant period ($P = .01$) (Figure 1). *E. coli* represented 47% of early GNR infections and decreased thereafter, whereas *Pseudomonas* spp. and *Klebsiella-Enterobacter-Serratia* (KES) were more frequently observed after engraftment. Three *Salmonella* spp. BSIs were diagnosed in the late or very late period. No significant changes occurred during the observation period.

Antibiotic susceptibility of the different isolates is shown in Table 3. Briefly, 67% of *E. coli* were resistant to fluoroquinolones and 77% were ESBL producers. The proportion of *Pseudomonas* spp. with multidrug resistance and carbapenem resistance was 21%. Antibiotic susceptibility did not change over the observation period but was different according to the post-transplant period. Fluoroquinolone resistance was 70% in the early period and 25% afterward ($P < .001$). Multidrug resistance was present in 83% of isolates in the early period and 33% afterward ($P < .001$). Carbapenem resistance was 30% before day +100 and 9% afterward ($P < .03$).

Fungemia

Fourteen of 15 fungal BSIs (13 single, 2 polymicrobial) were due to *Candida* spp. *C. krusei* was isolated in 6 episodes, *C. parapsilosis* in 3 episodes, and *C. tropicalis* and *C. glabrata* in 2 occasions each. *C. albicans* was only isolated in 1 case. Eleven fungal BSIs occurred during neutropenia.

Outcome of BSIs

Mortality according to the causative microorganism and transplant period is detailed in Table 4. The overall crude mortality rate was 12% at day 7 and 23% at day 30. Regarding BSI etiology, the 7-day mortality rate was higher for GNRs (18%) compared with GPB (7%), fungi (8%), and polymicrobial (8%) infections ($P = .03$). Mortality rates were not statistically different for CoNS and other GPB at day 7 (9% and 0%, respectively; $P = .3$) and at day 30 (19% and 25%, respectively; $P = .8$). Within GNRs, 7-day and 30-day mortality rates were highest for *Acinetobacter* spp. (50% and 88%), followed by *S. maltophilia* (25% and 38%), KES (25% and 25%), *Pseudomonas* spp. (17% and 25%), and *E. coli* (7% and 10%) ($P = .03$ and $P = .002$ for 7-day and 30 day mortality rates, respectively). Differences in mortality were also observed according to carbapenem susceptibility. Mortality rates at day 7 and day 30 for BSI caused by GNRs resistant or susceptible to carbapenem were 39% and 12% ($P = .02$) and 61% and 18% ($P < .001$), respectively. Mortality rate did not change significantly in the different post-transplant phases or throughout the observation period.

DISCUSSION

This study shows that BSI, the most common severe complication after myeloablative UCBT, was more frequently observed in patients receiving cord blood units with lower $CD34^+$ and $CD8^+$ cell doses. In addition, early BSI was also

Table 3
Antibiotic Resistance in Gram-Negative Isolates

Organism	Resistant to Fluoroquinolone	Resistant to Third-Generation Cephalosporin*	Resistant to Piperacillin-Tazobactam	Resistant to Carbapenem	Resistant to Aminoglycoside	ESBL Producers	MDR Bacteria
E. coli	21 (67)	7 (23)	3 (10)	0 (0)	0 (0)	24 (77)	23 (74)
Pseudomonas spp.	6 (21)	5 (17)	4 (14)	6 (21)	3 (11)	—	6 (21)
Klebsiella spp.	0 (0)	1 (25)	2 (50)	0 (0)	1 (25)	2 (50)	2 (50)
Enterobacter-Serratia spp.†	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
S. maltophilia	4 (36)	1 (9)	—	—	—	—	11 (100)
Acinetobacter spp.	10 (100)	10 (100)	10 (100)	7 (70)	4 (40)	—	10 (100)
Salmonella spp.	0 (0)	0 (0)	0 (0)	0 (0)	—	0 (0)	0 (0)

MDR indicates multidrug resistant.
Values are number of cases with percents in parentheses.
* In *Pseudomonas* spp. or *S. maltophilia* susceptibility to ceftazidime.
† In *Serratia* spp. tobramycin was not evaluated.

found to be a negative predictor of neutrophil recovery and 100-day NRM. We observed predominance of GNRs that were associated with a higher mortality rate.

This retrospective, single-center study included a large series of adults with a variety of hematologic malignancies, although most had acute leukemia, that received UCBT over a long period of time (almost 15 years). Patients received a relatively homogeneous conditioning regimen and supportive care, including infectious prophylaxis, empirical treatment of febrile neutropenia, and procedures for blood sample extraction and processing. Unlike all previous studies that focused on BSI after UCBT [5-7], all patients were followed throughout the observation period and information regarding BSIs was collected beyond the early post-transplant period. This allowed us to analyze differences in epidemiology at different time points. Because microbiologic epidemiology may vary significantly over time and in different institutions, some of the data should be interpreted with caution and may only be applicable in this specific setting.

We confirmed previous finding suggesting that early BSI was an independent risk factor for NRM in adults undergoing UCBT [7]. Interestingly, we also found early BSI to be a strong and independent predictor of neutrophil recovery, as a time-dependent covariate. These findings highlight the importance of preventing early bacterial infection. The exact mechanism by which BSI can interfere with engraftment is unknown. We could hypothesize that bacterial endotoxins or host cytokines could have hampered homing and differentiation of cord blood progenitor cells. However, the possibility that early BSI could be a surrogate marker of graft cellularity cannot be ruled out.

BSIs were frequent complications after UCBT with a cumulative incidence of 42% at day 100 and 54% at 4 years, which was in line with previously reported rates using other stem cell sources [2,20]. Although UCBT is characterized by a longer time to neutrophil recovery, to date this procedure has not been consistently associated with an increased risk of bacterial infections [4,21]. Two studies from the University of Tokyo reported a cumulative incidence of BSIs of 32% at day 100 for reduced intensity [5] and 12% at day 30 for myeloablative conditioning [6]. An additional study from the Japan Cord Blood Bank Network [7] reported a cumulative incidence of 21% at day 100. The difference is likely due to local epidemiology and transplant, infectious, and laboratory procedures. However, the incidence of BSI could have been underestimated when focusing on early infections. It should be noted that in our study around 60% of BSI episodes were observed after neutrophil recovery and around 30% after day +100. Regarding the analysis of risk factors for BSI, to date no risk factors have been identified in the UCBT setting except for an increased incidence in the adult population. We describe here for the first time the impact of cord blood unit cell dose, in terms of $CD34^+$ cells and $CD8^+$ cells, on the risk of BSI. This finding is expected because we previously described the importance of these markers on neutrophil recovery [11].

Table 4
Mortality Rate after BSI Episodes According to the Etiologic Agent

Organism	Number of Episodes	7-Day Mortality N (%)	30-Day Mortality N (%)
Total	189	23 (12)	44 (23)
Gram-positive	76	5 (7)	16 (21)
Gram-negative	87	16 (18)	24 (28)
Other Gram-	11	2 (18)	2 (18)
E. coli	28	2 (7)	3 (10)
Pseudomonas spp.	24	4 (17)	7 (29)
KES	8	2 (25)	2 (25)
S. maltophilia	8	2 (25)	3 (38)
Acinetobacter spp.	8	4 (50)	7 (88)
Fungi	13	1 (8)	2 (15)
Polymicrobial	13	1 (8)	2 (15)

Except for a small predominance of GPB before engraftment, which was mainly due to CoNS, GNRs were more frequently observed in all the remaining post-transplant periods and overall. This is in contrast to most published studies demonstrating that gram-positive organisms were responsible for most BSIs after allo-HSCT [20,22,23], including UCBT [5-7,9]. Significant variations in epidemiology or antibiotic susceptibility over time were not found, except for an increase of *Streptococcus viridans* and *Enterococcus* species after 2007. However, findings on the different transplant periods merit some attention. The early post-transplant period was characterized by the presence of *S. aureus*, *Streptococcus viridans*, *E. coli*, and *Candida* species that were replaced by *Enterococcus* species, *Pseudomonas* spp., and KES in the postneutropenic phase. Of note, 3 *Salmonella* spp. were isolated after day 100.

Regarding antibiotic susceptibility, isolates were frequently resistant. A high rate of fluoroquinolone resistance was mainly observed during the early post-transplant period, corresponding with the widespread use of ciprofloxacin prophylaxis. Whether or not this policy should be changed because of the high rate of resistance is a difficult matter, because the efficacy of this procedure was not evaluated in the present study and the number of quinolone-sensitive BSIs that were potentially prevented is not known. This pattern of resistance is particularly important to guide the choice of empirical antibiotic treatment. The observation of a high rate of

ESBL-producer enterobacteria, carbapenem-resistant *Pseudomonas* strains, and frequent isolates of *S. maltophilia* and *Acinetobacter* spp. may reflect the high-risk population included in this study. Most patients had received multiple prior high-dose chemotherapy cycles, with prolonged neutropenia, and high exposure to antimicrobial treatment and were in need of an urgent transplant. Knowledge of this epidemiology is crucial to design protocols and strategies for antibiotic prophylaxis and empirical antimicrobial treatment of fever in UCBT recipients.

Evaluation of outcome after BSI and the detection of prognostic factors affecting mortality are important matters. Early crude mortality rate was analyzed in an effort to overcome bias and discriminate from other variables that can influence mortality in the allo-HSCT setting. Seven-day and 30-day mortality rates were similar to those previously reported after high-risk transplants [21]. As expected, mortality was higher for GNRs compared with GPB, and this difference remained if CoNS were excluded from the analysis (data not shown). The main contributors to mortality were the etiology of BSI and resistance to carbapenem, which was used as empirical therapy of febrile neutropenia.

In conclusion, we highlighted the importance of BSI after myeloablative UCBT in adults with hematologic malignancies, particularly with the use of cord blood units with low cellularity. Interestingly, early BSI not only increased NRM but hampered neutrophil recovery. These findings emphasize the importance of prevention of bacterial infections during conditioning and the very early post-UCBT period.

ACKNOWLEDGMENTS

The authors thank Shirley Weiss and David Pellicer for data collection and management.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: J.S., M.S., and G.F.S. conceived the study and interpreted the data. J.S., I.C., and M.S. wrote the manuscript. J.S. performed the statistical analyses. E.M.G.-B. and J.L.L performed microbiologic tests and collected microbiologic data. I.C., E.M.G.-B., M.A., J.R., P.M., I.L., I.J., J.M., F.L., M.J.A., A.L., F.M., J.L.L., and M.S. reviewed the manuscript and contributed to the final draft.

Financial disclosure: The authors have nothing to disclose.

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